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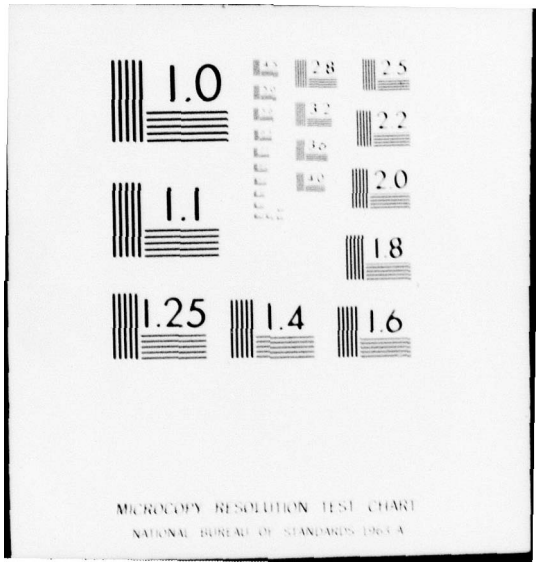
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TECHNICAL REPORT ARCSL-TR-78025

COMPARISON OF CELLULOSE AND POLYCARBONATE MEMBRANE FILTERS FOR DETECTION OF MICROBIAL CELLS

by

Michael Shapel
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March 1978

US ARMY ARMAMENT RESEARCH AND DEVELOPMENT COMMAND
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Membrane filters	Bacteria	Chemical indicator
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

(U) Two types of membrane filters, cellulose ester and polycarbonate, were evaluated for their application in the chromophoric detection of bacteria based on reduction of an oxidation-reduction indicator (resazurin). The cellulose filters gave superior results attributed to their depth-type structure which provided a greater surface area for adsorption of nutrient media. Both filters retained 99.9% of all bacteria. Resazurin reduction was detected for a hundredfold

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20. ABSTRACT (Contd)

lower concentration of bacteria on cellulose filters than on the polycarbonate filters. These results were consistent with better accessability of substrate to the indicator on cellulose filters, thereby enabling more rapid and sensitive color changes.

PREFACE

The work described in this report was authorized under Project 1L762711AD34, Physical Defense Against Biological Attack. The work was started in February 1977 and completed in July 1977.

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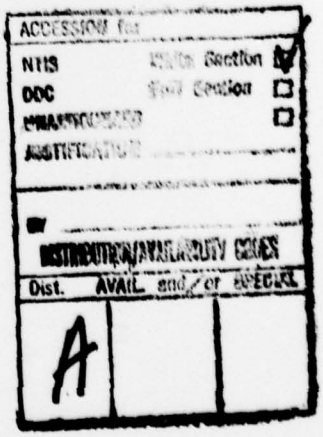

CONTENTS

COMPARISON OF CELLULOSE AND POLYCARBONATE MEMBRANE FILTERS FOR DETECTION OF MICROBIAL CELLS

I. INTRODUCTION.

Two types of membrane filters were examined for characteristics that would have minimal inhibitory effects on collected bacteria and would allow rapid reduction of resazurin. The use of resazurin as an oxidation reduction indicator for measuring bacterial dehydrogenase activity has been well documented.[1-4] In these dehydrogenase reactions, the resazurin changes from blue to pink (resorufin) to colorless (hydroresorufin). The colors produced are satisfactory for viable detection of bacteria.

The criteria used for developing a membrane filter technique to detect viable bacteria are: (1) the bacteria should be efficiently retained by the filter, (2) the reduction product must be of sufficient color contrast when viewed against the filter background, and (3) bacterial dehydrogenases should not be affected by the filter material.

The structure of the polycarbonate filters used in this study differs materially from that of cellulose filters and the filtration properties differ correspondingly.[5] The polycarbonate filters are unique in being thin ($\leq 10\ \mu m$) and flat with a smooth surface; the cellulose membrane filters are thicker ($>100\ \mu m$) with a comparatively rough surface.[5-7] The study reported here consisted of comparison of resazurin activity for four species of bacteria collected on both types of filters.

II. MATERIALS AND METHODS.

A. Membrane Filters.

This investigation was limited to a comparison of two commercial brands of membrane filters differing widely in composition and structure: The Millipore white (cellulose) filter (HAWP013), composed of pure and inert esters of cellulose with 0.45-μm porosity and sterilized with ethylene oxide (Millipore Corporation, Bedford, Massachusetts), and the Nuclepore (polycarbonate) filter, composed of polycarbonate (N040CPR 01300) with 0.40-μm porosity and sterilized in an autoclave (Nuclepore Corporation, Pleasanton, California).

B. Stock Resazurin Solution.

Resazurin solution was prepared by adding one resazurin tablet (National Aniline Division of the Allied Chemical and Dye Corporation, New York, New York), which contained 10.8 mg of dye, to 100 ml of 0.85% saline prepared in pyrogen-free water. The resazurin tablet was dissolved at room temperature using a magnetic mixer. Following sterilization by filtration through a membrane filter (0.20-μm pore size, Nalge Corporation), the solution was dispensed aseptically into a sterile amber-colored bottle and stored at room temperature. A fresh stock solution was prepared every 30 days.

C. Bacteria.

The filters were compared by testing with pure cultures of several species of bacteria as reference organisms (obtained from the American Type Culture Collection). *Alcaligenes faecalis* (0.5 by 1.0 to 2 microns), *Aerobacter aerogenes* (0.5 to 0.8 by 1.0 to 2.0 microns), *Serratia marcescens* (0.5 by 0.5 to 1.0 micron), and *Proteus mirabilis* (0.5 to 0.6 by 1.0 to 3.0 microns)[8] were used. Each species was streaked on nutrient agar to determine purity. A characteristic colony was selected and cultured in a growth medium at 37°C. The culture was stored at 4°C to provide an inoculum source for fresh test cultures used in this study.

D. Plating and Growth Media.

Plating and broth media for routine colony counts and broth cultures were prepared from commercially available dehydrated media. Nutrient agar was obtained from DIFCO Laboratories, Inc., Detroit, Michigan. The sterilized nutrient agar was cooled to 50°C in a water bath and 15- to 20-ml portions were dispensed into sterile plastic Petri dishes (15 by 100 mm). The plate surfaces were allowed to dry for 24 hours at room temperature and the plates were stored at 4°C until use. The broth media used for growth and membrane filter testing consisted of lactalbumin hydrolysate (DIFCO) broth supplemented with yeast extract and peptone (LAHYEP). The LAHYEP medium contained 1 liter of Hanks balanced salts in demineralized water (grams per liter: $CaCl_2$, 0.14; KH_2PO_4, 0.06; $MgSO_4$, 0.2; glucose, 1.0; NaCl, 8; KCl, 0.4; $Na_2HPO_4 \cdot H_2O$, 0.06; 5 grams of lactalbumin hydrolysate, 1 gram of yeast extract, and 0.5 gram of peptone). The medium was adjusted to pH 7.0 with 0.1 N NaOH and sterilized by passing through a sterile 0.20-μm membrane filter (Nalge Corporation); 50 ml of sterile medium was aseptically dispensed into each of the required number of sterile 125-ml Erlenmeyer flasks.

E. Preparation of Bacterial Test Cultures.

Fifty-milliliter volumes of prewarmed LAHYEP broth in 125-ml Erlenmeyer flasks were inoculated with 1 ml of the stock inoculum and incubated 24 hours at 37°C. The resulting cultures were usually at the peak of the growth cycle and consisted entirely of vegetative cells which were used immediately for comparison of resazurin reduction on the two types of filters.

F. Colony Counts.

To determine the viability of stock cultures and the growth response of bacteria in LAHYEP broth, serial tenfold dilutions were made in LAHYEP broth and plated on nutrient agar plates. The retention efficiency of filters was also determined by plating aliquots of filter effluent.

G. Membrane Filter Technique for Resazurin Reduction Assay of Bacteria.

The filters were inoculated by filtering a suspension of the desired number of cells in 1 ml of sterile LAHYEP broth prepared by serial dilution of the culture in the broth. The filtration was done in Swinney holders as follows: cellulose filters were placed with the side facing the top of the package oriented in the upstream direction (i.e., toward the syringe); polycarbonate filters were used with the shiny side up. Pressure was applied to the syringe plunger to force 1 ml of suspension through the filter (effective filtering area of 0.8 cm^2). After removing the syringe, a 10-ml Luer/Lok syringe was attached to force through the remaining fluid. The Swinney holder was

disassembled and the filter was removed from the top of the gasket using forceps with unserrated tips. The filters, with the inoculated side up, were placed in concavities of glass slides. Slides measuring 2 by 4 inches with six concavities and lubricated with vegetable shortening (Crisco) were used. Approximately 0.20 ml of a mixture of one part stock resazurin and eight parts of LAHYEP broth containing 0.0012 mg/ml of resazurin was added. The slides were transferred to Petri dishes containing wet sponges and incubated at 37°C. In all testing, controls consisted of filters through which uninoculated LAHYEP broth was filtered. The time of a positive reaction was recorded when resazurin was reduced from blue to purple to pink (resorufin).

III. RESULTS.

The results recorded in table 1 show that the cellulose membrane filters were superior to polycarbonate filters for detection of bacteria by resazurin reduction. A comparison of experimental results for both filters showed that, with cellulose membrane filters, resazurin was more rapidly reduced and lower cell numbers could be detected. The latter results were obtained with all four species tested at total cell concentrations of 10^6 and 10^5. The lowest cell concentration detected with polycarbonate filters was 10^7. This was an approximate loss of two logs. Concentrations of 10^7 cells consistently reduced resazurin more rapidly on cellulose filters. Similar results for both filters could only be obtained at the 10^8 level. In general, greater sensitivity and rapidity of reaction were obtained with cellulose filters.

Table 1. Comparison of Resazurin Reduction by Microbial Cells Concentrated by Filtration on Polycarbonate and Cellulose Filters

Organism [a]	Colony-forming units per milliliter on nutrient agar	Time [b] required for color change [c]			
		Polycarbonate filter		Cellulose filter	
		Purple	Pink	Purple	Pink
		min		min	
Alcaligenes faecalis	1.1×10^8	4	8	4	8
	1.1×10^7	25	120	9	20
	1.1×10^6	NR [d]	NR	45	65
	1.1×10^5	NR	NR	105	145
Aerobacter aerogenes	1.5×10^8	4	8	3	7
	1.5×10^7	80	150	13	23
	1.5×10^6	NR	NR	70	85
	1.5×10^5	NR	NR	120	150
Serratia marcescens	1.2×10^8	3	10	3	8
	1.2×10^7	21	95	10	40
	1.2×10^6	NR	NR	88	115
	1.2×10^5	NR	NR	125	175
Proteus mirabilis	1.4×10^8	5	10	4	9
	1.4×10^7	30	100	16	37
	1.4×10^6	NR	NR	75	111
	1.4×10^5	NR	NR	89	197

[a] Viable organisms suspended in LAHYEP.

[b] Values are means of duplicate culture tubes given in minutes.

[c] Only purple to pink (resorufin) coloration was considered positive for detection.

[d] No reduction.

The data in table 2 on retention of bacteria show that cellulose filters did not retain more bacteria than polycarbonate filters. In addition, there were no significant differences for bacterial retention among membrane filter lots.

Table 2. Efficiency of Bacterial Retention by Polycarbonate and Cellulose Filters

Bacteria	Percent retained by filter [a]	
	Polycarbonate (0.4-μm porosity)	Cellulose (0.45-μm porosity)
S. marcescens (0.5 by 0.5 to 1.0 μm)	99.89 [b]	99.92
A. faecalis (0.5 by 1.0 to 2.0 μm)	99.90	99.93

[a] Polycarbonate lots 81C6425 and 81F6839; cellulose lots HA28762-1 and HA29553-1.

[b] Each value represents the mean for the two lots of each filter type.

IV. DISCUSSION.

This study indicates that there are significant differences in the performance characteristics of polycarbonate and cellulose filters that are probably accounted for by differences in matrix characteristics as is evident in scanning electron microscope illustrations in figures 1 and 2. The cellulose filter is a depth-type filter (figure 3) which retains not only bacteria larger than its pore size on the surface but also particles smaller than rated pore size by pore restriction and within the twisting form of the matrix. This property permits greater adsorption by cellulose membrane filters. Conversely, the surface area of the polycarbonate filters is only one-thirtieth that of equal diameter and porosity of cellulose filters, as determined by Brunauer, Emmett, and Teller surface area measurements.[9] Adsorption is, therefore, of a much lower order. For example, it was demonstrated by Rescelli, et al.,[10] in an equilibrium dialysis experiment, that nonspecific adsorption of antibody was less than 8% for a polycarbonate membrane and almost 60% for a cellulose membrane. Figure 3 shows that polycarbonate membranes have round pores distributed at random over a thin and extremely smooth and flat surface on both sides. The low adsorption power of polycarbonate filters[11] for nutrient substrates and bacteria may be attributed to strictly physical properties. These include: straight-through pores, thin film support (one-tenth the thickness of cellulose membranes), and smooth surface. Furthermore, the smooth nonwhite membrane surface provides a lower degree of color contrast with reduced resazurin and makes reading of color changes more difficult.

The results obtained in this study indicate that the evaluation of membrane filters for collecting bacteria and determining viability characteristics should not be based on a single high concentration of bacterial cells. A profile of decreasing bacterial concentrations must be used to determine the efficiency of a given filter. For example, in table 1, polycarbonate filters are ineffective for bacterial cell numbers below 10^7.

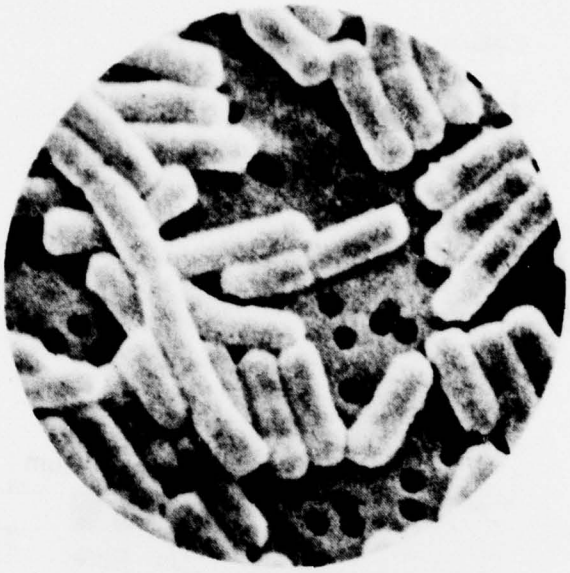

Figure 1. Scanning Electron Micrograph of *Bacillus subtilis* on a 0.4-μm Uni-Pore Polycarbonate Membrane Filter (5,000 X)

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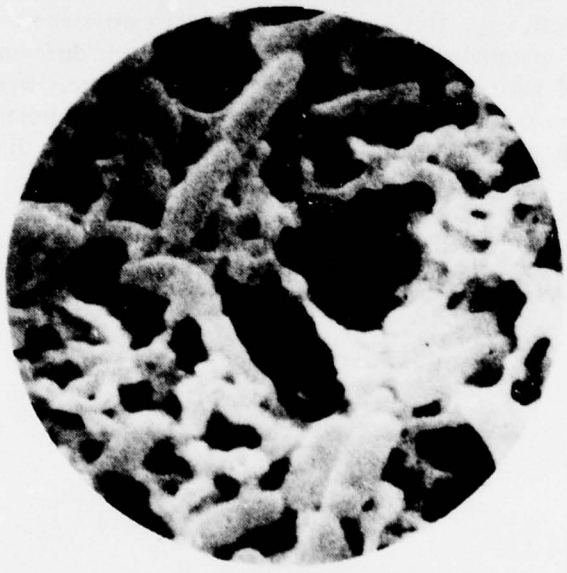

Figure 2. Scanning Electron Micrograph of *Bacillus subtilis* on a 0.45-μm Cellulose Membrane Filter (5,000 X)

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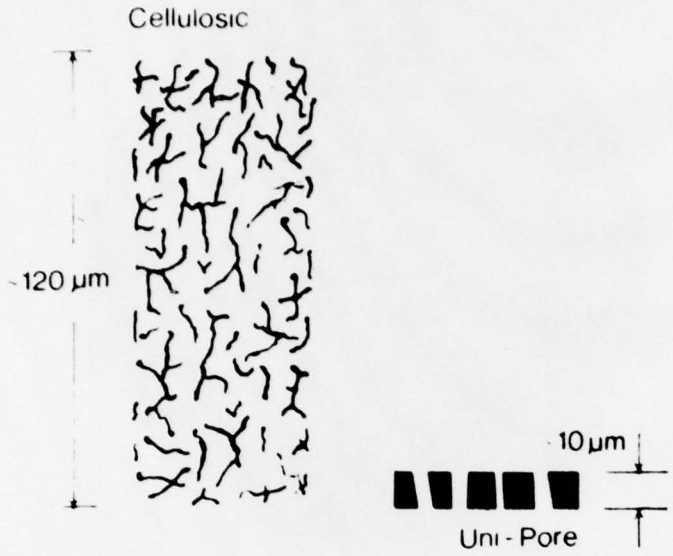

Figure 3. Graphic Comparison of Cross-Section of Uni-Pore Membrane and Cellulosic Filter

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The oxidation-reduction activities of bacteria on both polycarbonate and cellulose membrane filters in the presence of nutrient substrate and resazurin show marked differences in time of reaction and sensitivity. This can be attributed to differences in internal structure and adsorption properties of material for both filters. No significant differences in bacterial retention between cellulose (0.08% loss) and polycarbonate (0.1% loss) filters were evident. The slight loss might be attributed to morphological variants smaller than the rated pore size. In addition, pressure applied to the syringe plunger could have forced through bacteria with dimensions close to the pore size of the filter.

The present study indicates that cellulose filters are preferable for collecting and detecting bacteria by the resazurin method especially for lower concentrations of cells.

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